

AL-AZHAR UNIV CAIRO (EGYPT) DEPT OF CLINICAL PATHOLOGY F/6 6/13
LYSOGENY AND BACTERIOGINOGENY IN SALMONELLA, SHIGELLA, BACILLUS--ETC(U)
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BACTERIAL TYPING PROJECT

Clinical Pathology Department

Faculty of Medicine

Al-Azhar University

Nasr City, Cairo R. A. E.

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OFFICE OF NAVAL RESEARCH

Contract Number N00014 - 73 - C - 0009, NRI36 - 931

Annual Report Number I
(April 1973 - March 1974)

"Lysogeny and Baeteriocinogeny in Salmonella, Shigella, Bacillus
Pyocyanus and Neisseria meningitidis"

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"Lysogeny and Bacteriocinogeny in Salmonella, Shigella, Bacillus Pyocyaneus and Neisseria meningitidis"

I. - Salmonella

A. - Salmonella typhi

There were 26 locally isolated strains of S. typhi selected for phage typing. All were "V1" positive.

Phage typing of S. typhi strains was performed by the basic method of Craigie & Felix (1947) with standard phages as described by Nicolle et al (1963).

Analysis of the phage typing patterns of this group revealed 7 phage types including the non-typable strains. The distribution of the phage types is shown in the following table.

Phage types of Salmonella typhi

<u>Phage type</u>	<u>No. of strains</u>	<u>Percentage</u>
C ₁	8	30.8
aleonosenstive	6	23.1
D ₁	4	15.3
A	2	7.7
L ₂	2	7.7
I+IV	2	7.7
non-typable	2	7.7

Biochemical and Colicin typing of these organisms are being performed. Interpretation of the result will be reported in the coming semi-annual report.

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B. - Salmonella paratyphi A

Phage typing of 48 locally isolated *S. paratyphi A* strains following the method of Banker (1955) resulted in 4 groups as shown in the following table.

Phage typing of Salmonella paratyphi A

<u>Phage type</u>	<u>No. of strains</u>	<u>Percentage</u>
2	24	50.0
1	20	41.6
4	2	4.2
non-typable	2	4.2

Complementary phage typing of *S. paratyphi A* (Hablas & Nicolle 1969) are being carried out on the collected *S. paratyphi A* phage type 1 strains.

II. - Shigella

A combined research work with Pediatrics Department, Ain-Shams Faculty of Medicine is being carried out to study the bacteriology of summer diarrhoea in children in the hope of isolation of different *Shigella* serotypes. The locally isolated *Shigella sonnei* strains will be phage typed.

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III. - Bacillus pyocyaneus

Isolates of Bacillus pyocyaneus from different human sources (cross hospital infections, middle ear diseases, chronic prostatitis and urinary tract infections) are being collected. These isolates will be serologically, pyocin and phage typed.

IV. - Neisseria meningitidis

Due to the delay in the accomplishment of some Pro-forma Invoices for Neisseria meningitidis antisera and specific enrichment media, research on Neisseria meningitidis will not start until next season. Isolates from human carriers and cerebro-spinal meningitis patients will be employed.

According to the economic policy recently adopted by the Egyptian government, it has been possible for various Pro-forma Invoices to come to the execution stage. This has permitted an easier inflow of laboratory equipment, antisera, and chemicals.

R. Hablas.

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References:

- Dankor, D.D. 1955. Paratyphoid-A phage typing. Nature 175: 109.
- Groglis, J.; Felix, A. 1947. Typing of typhoid bacilli with VI bacteriophage. Lancet 1 (252): 823 - 827.
- Hablas, R.; Nicolle, P. 1959. Phage typing of Salmonella paratyphi A by the method of Dankor and complementary phage typing of phage type 1. C.R. Acad. Sc. (Paris) 268:2521 - 2524. (In French).
- Nicolle, P.; Brunet, J.; Livornoau, J. 1963. Phage typing of Salmonella typhi. Rev. Hyg. et Med. Soc. 12:7 - 43. (In French).
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Contract Number N00014 - 73 - C - 0009, NRL36 - 931

Annual Report Number II

(April 1974 - March 1975)

"Lysogeny and Bacteriocinogeny in Salmonella, Shigella, Bacillus
Pyocyaneus and Neisseria meningitidis"

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Nasr City, Cairo R. A. E.

"Lysogeny and Bacteriocinogeny in Salmonella, Shigella, Bacillus pyocyaneus and Neisseria meningitidis"

I.- Salmonella

A.- Salmonella typhi

A total of 134 strains of Salmonella typhi, isolated from Cairo and Alexandria¹ for phage typing, proved "Vi" positive.

These Salmonella typhi isolates were phage typed according to Craigie and Felix (1947) method, taking phage typing technique described by Nicolle et al (1963) as standard.

The frequencies of the 20 phage types revealed by analysis of these isolates are demonstrated in the following table.

¹ Alexandria strains are forwarded by Dr. A. Lackany, Director, "Epidemiological and Bacteriological Study of Typhoid Fever in Alexandria" Project.

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Phage types of Salmonella typhi

<u>Phage type</u>	<u>No. of strains</u>	<u>Percentage</u>
D ₁	22	16.3
C ₁	21	15.7
I + IV	19	14.1
aliénosensible	16	11.9
A	14	11.0
40	9	6.7
D ₈	7	5.2
N.T.	7	5.2
C ₁₀	3	2.2
46	3	2.2
D ₉	2	1.5
E ₂	2	1.5
L ₂	2	1.5
C ₁₁	1	0.8
E ₄	1	0.8
F ₃	1	0.8
G ₁	1	0.8
J ₁	1	0.8
H	1	0.8
28	1	0.8
TOTAL	134	100.0%

Christensen biochemical typing of these organisms has revealed 114 strains of chemotype I and 20 of chemotype II.

As for colicin typing, 121 isolates were found colicin negative, while the remaining 13, among which 9 were phage type 40 and 4 were phage type I + IV, proved colicin positive.

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B.- Salmonella paratyphi A

45 Salmonella paratyphi A strains were isolated from the same issues as in Salmonella typhi, these strains were phage typed according to the method of Banker (1955), giving rise to the following results:

Phage typing of Salmonella paratyphi A

<u>Banker Phage type</u>	<u>No. of strains</u>	<u>Percentage</u>
1	33	73.4
2	8	17.8
4	2	4.4
Non-typable	2	4.4
TOTAL	45	100.0%

Salmonella paratyphi A strains of phage type 1, adding to 33, have been subjected to complementary phage typing following Hablas and Nicolle (1969). Results are as follows:

<u>Hablas Phage type</u>	<u>No. of strains</u>	<u>Percentage</u>
a	24	72.7
c	4	12.1
e	2	6.1

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II.- Bacillus pyocyaneus

A combined research work in cooperation with the Urology Department, Al-Azhar Faculty of Medicine, aiming to display the relationship between Bilharziasis and Bacillus pyocyaneus in urinary tract infection which is common in our Bilharzial patients, was carried out.

Samples of urine, tissue and calculous were collected from different anatomical levels of urinary tract of 80 Bilharzial patients, and 20 non-Bilharzial as a control.

Among the Bilharzial patients, 18 gave rise to the isolation of 31 strains of Bacillus pyocyaneus.

Isolates were serologically typed after their morphological and biochemical identification. The results were:

<u>Serotype</u>	<u>No. of strains</u>	<u>Percentage</u>
12	6	19.3
1	5	16.1
11	3	9.6
13	3	9.6
2	2	6.5
5	2	6.5
7	2	6.5
10	2	6.5
Non-agglutinable	4	12.9
Polyagglutinable	2	6.5
TOTAL	31	100.0%

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Pyocin typing of these isolates is being achieved and will be forwarded.

Effect of Antibilharzial Treatment

Treatment of 8 Bilharzial patients, suffering from non-obstructive lesions and who proved to have Bacillus pyocyaneus with only tartar emetic 6% and without any chemotherapy, resulted in clearance of the infecting organisms from their urinary tracts. Follow up samples are being examined.

10 patients with obstructive Bilharzial lesions who proved to have Bacillus pyocyaneus in their urine showed no response to the combination of tartar emetic and chemotherapy, and the Bacillus pyocyaneus only cleared after surgical interference and regaining the patency of the urinary tract. Follow up samples of urine are examined for clearance of Bacillus pyocyaneus.

18 out of 20 non-Bilharzial patients, subjected to the same bacteriological investigations, showed no growth for Bacillus pyocyaneus.

The two remaining patients proved to have Bacillus pyocyaneus in specimens collected from them during surgical interference. The first of these yielded a positive culture in urine from his pyonephrotic kidney, while the second gave rise to Bacillus pyocyaneus growth by culturing a biopsy from the bed of the stone.

N.B. Detailed findings of this research work entitled "Study of Pyocyaneus Infection of the Urinary Tract in Urological Bilharzial Patients", will be forwarded upon publication of the thesis.

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III.- Neisseria meningitidis

19 strains of Neisseria meningitidis Group A isolated from patients' throat or cerebro-spinal fluid cultures were examined against each other for lysogeny. No lysogenic effect could be proved. Research for lysogeny are being tried against other serogroups of Neisseria meningitidis and other related species of Neisseria.

The Neigon and Flow Laboratories, Inc., have been kind enough to send us Neigon JEMBEC plates and Neigon CO₂ tablets, free of charge. The application of this new Neigon system may be very useful in the field isolation of different members of the Neisseria species.

References:

- Banker, D.D. 1955. Paratyphoid-A phage typing. Nature 175:309.
- Craigie, J.; Felix, A. 1947. Typing of typhoid bacilli with Vi bacteriophage. Lancet 1 (252): 823 - 827.
- Hablas, R.; Nicolle, P. 1969. Phage typing of Salmonella paratyphi A by the method of Banker and complimentary phage typing of phage type 1. C.R. Acad. Sc. (Paris) 268:2521-2524. (In French).
- Nicolle, P.; Prunet, J.; Diverneau, G. 1963. Phage typing of Salmonella typhi. Rev. Hyg. et med. Soc. 12:7-43.(In French).

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Consultant's Visit

We have arranged for our off-campus consultant, Dr. J.F. Vieu, Head of Bacteriophage Service, Pasteur Institute, France, to visit our Project.

Dr. Vieu had spent in Egypt a two-week period during which he held two lectures entitled "Pseudomonas aeruginosa as a causative agent in cross hospital infection", which was held in Al-Hussein Hospital, and "Epidemiological Markers of Salmonella typhi and Salmonella paratyphi B; (Principle, stability and variation)", which has been performed in NAMRU-3.

Our off-campus consultant has also attended some practical work in our laboratories, held discussions with the project investigators and witnessed our techniques.

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Theses Supported in Part by the Project

- | | |
|--------------------------------|--|
| 1. Dr. Mohamed Mahmoud Ismail | "Establishment of Scheme of Phage Typing by Study of Lysogeny in Salmonella gallinarum-pullorum Group Isolated from Different Sources and Localities". |
| 2. Dr. Ezz El Rigal Khamis | "Correlation Between Enteric Infection in Egypt and Serological and Other Related Laboratory Findings." |
| 3. Dr. Hussein Amr | "Study of Pyocyanus Infection of the Urinary Tract in Urological Bilharzial Patients". |
| 4. Dr. Ibrahim Mohamed Shabana | "Flora of Nasopharynx in Allergic and Bacterial Rhinitis with Special Stress on Neisseria Species". |
| 5. Dr. Elham Metawie | "Serology in Diagnosis of Meningococcal meningitis". |
| 6. Dr. Mohamed Ali Saleh | "Clinical, Bacteriological and Immunological Studies on Chronic Tonsillitis in Egyptian Students". |
| 7. Dr. Ahmed Mohamed Salem | "Bacteriological Study of Nasopharyngeal Infection and Chronic Discharging Ear". |
| 8. Dr. Mohamed Mustapha Saad | "Incidence of Pathogenic Organisms in the Normal Flora of the Neonates External Ear and Nasopharynx in Comparison to Their Contacts". |

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| 9. Dr. Fakhry Hassaballah | "Relation between Chronic Tonsillitis as a Septic Focus and Chronic Prostatitis". |
| 10. Dr. Abdalla El Sheikh | "Peritonitis in Egypt". |
| 11. Dr. Safeya Abul Makarem | "Bacterial Species in Septic Abortion in Relation to the Blood Coagulation". |
| 12. Dr. Mustapha Fawi Rabie | "Study of Antibiotic Resistance in E. coli and Possible Transfer to Intestinal Pathogens". |
| 13. Dr. Mohamed Bassiouni | "Clinico-Pathological Studies in Male Asswanians Affected by Bilharziasis after High Dam". |

N.B. The necessary research works of the here-mentioned theses coincide with the isolation from different sources , and typing of the various microorganisms of interest to our Project.

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Contract Number NO0014 - 73 - 0009, NR 136 - 931

Annual Report Number III

(April 1975 - March 1976)

"Lysogeny and Bacteriocinogeny in Salmonella, Shigella, Bacillus
pyocyaneus and Neisseria meningitidis"

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"Lysogeny and Bacteriocinogeny in Salmonella, Shigella,

Bacillus pyocyaneus and Neisseria meningitidis"

I.- Salmonella

A.- Salmonella typhi

Phage typing of 54 strains of Salmonella typhi isolated in Cairo and Alexandria in the amounts of 26 and 28 respectively, was accomplished during the current year by use of the same method of Craigie and Felix (1947), yielding the following results:

Cairo =====		
<u>Phage Type</u>	<u>No. of Strains</u>	<u>Percentage</u>
Aliénosensible	6	23.07%
I+IV	5	19.22%
N.T.	3	11.53%
A	2	7.69%
46	2	7.69%
C ₁	1	3.85%
D ₁	1	3.85%
D ₅	1	3.85%
E	1	3.85%
E ₁	1	3.85%
E ₂	1	3.85%
G ₁	1	3.85%
40	1	3.85%
TOTAL	26	100.00%

Alexandria
=====

<u>Phage Type</u>	<u>No. of Strains</u>	<u>Percentage</u>
I+IV	7	25.00%
N.T.	7	25.00%
A	4	14.29%
C ₁	4	14.29%
Aliénosensible	3	10.71%
C ₁₁	1	3.57%
E ₁₀	1	3.57%
46	1	3.57%
TOTAL	28	100.00%

The same strains have been investigated for biochemical and colicin Typing. The results showed that among Alexandria strains 15 were chemotype I and 13 chemotype II with 23 colicin-negative strains and 5 colicin-positive. As for strains collected from Cairo, 14 were chemotype I, and 12 chemotype II, with 25 colicin-negative strains and only one colicin-positive.

B.- Salmonella paratyphi A

Banker phage typing has been used for 75 Salmonella paratyphi A cultures, among which 43 were isolated in Cairo, and 32 in Alexandria. The results were:

Cairo
=====

<u>Phage Type</u>	<u>No. of Strains</u>	<u>Percentage</u>
1	33	76.75%
2	6	13.95%
4	2	4.65%
N.T.	2	4.65%
TOTAL	43	100.00%

Alexandria
=====

<u>Phage Type</u>	<u>No. of Strains</u>	<u>Percentage</u>
1	28	87.50%
2	2	6.25%
4	2	6.25%
TOTAL	32	100.00%

Besides, 190 strains of Salmonella paratyphi isolated in Indonesia, have been recently received from the U.S. Naval Medical Research Unit No.2, Djakarta Detachment, for phage typing.

Disregarding one rough strain which is not usually affected by phages, Banker phage typing has been used for the remaining strains, giving the following results:

NAMPU-2
Djakarta Detachment
=====

<u>Phage Type</u>	<u>No. of Strains</u>	<u>Percentage</u>
1	93	49.21%
5	87	56.03%
2	1	0.53%
4	1	0.53%
N.T.	3	1.59%
Atypical	4	2.11%
TOTAL	189	100.00%

Comparative interpretation of these results to the findings reached by phage typing of 168 strains in Egypt, during the last three years can be more appropriately featured in the following table:

Comparative Frequencies of *Salmonella paratyphi* A
in Egypt and Indonesia
=====

Phage Type	Egypt =====		Indonesia =====	
	No. of Strains	Percentage	No. of Strains	Percentage
1	114	67.86%	93	50.27%
2	40	23.81%	1	0.54%
4	8	4.76%	1	0.54%
5	-	-	87	47.03%
N.T.	6	3.57%	3	1.62%
TOTAL	168	100.00%	185	100.00%

We notice that phage type 1 is the most predominating in both areas. However, while phage type 2 comes second in prevalence in Egypt, phage type 5 occupies this 2nd order among Indonesian types of *Salmonella paratyphi* A despite its being a type of rare occurrence all over the world, according to the findings of Hablas and Nicolle (1969)¹.

This comparison is very useful as to the geographical distribution of *Salmonella paratyphi* A, which is one of our main interests.

¹Rifaat Hablas et Pierre Nicolle. 'Lysotypie de *Salmonella paratyphi* A par la méthode de Banker et lysotypie complémentaire inédite du lysotype 1'. C.R. Acad. Sc. Paris, t. 268, P. 2521-2524 (19 Mai 1969).

D.- Newly isolated Salmonella serotype in Egypt

For the first time in Egypt, we have recently isolated Salmonella havana (1,13,23 : f,g : -), a member of the Salmonella serogroup G2, from the stools of an intestinal bilharzial patient suffering from gastroenteritis.

The bacteriological diagnosis of this Salmonella culture has been confirmed by the International Salmonella Center, Institut Pasteur, Paris.

This Salmonella havana adds to the collection of Salmonella serotypes newly isolated from humans in Egypt mentioned in the work of Hablas et al (1973).²

The antibiogram of this Salmonella havana culture showed that it is multiple resistant to:

Tetracycline, Chloramphenicol, Streptomycin, Ampicillin, and Sulphanomide.

and, sensitive to:

Kanamycin, Nalidixic acid, Garamycin, Cephalothin, Neomycin, Colistine sulphate, Polymixin, and Trimethoprim.

Further study of the probability of the transfer of this resistance to other intestinal pathogens is being carried out.

²R. Hablas. W.P. Sanborn and K. Sorensen. 'Salmonella Serotypes Newly Isolated from Humans in Egypt. Tropical and Geographical Medicine. 26 (1974) 84-86.

E.- Salmonella typhi-murium

Twelve strains of Salmonella typhi-murium had been isolated in two outbreaks of gastro-enteritis due to consumption of meat from two emergency slaughtered sick camels. Phage typing of the 6 strains isolated from patients in the first outbreak (Shubra-Tana, Gharbia Governorate) proved to be phage type 41. On the other hand, the remaining 6 strains isolated from patients and camel meat in the second outbreak (Mashtoul El-Souk, Sharkia Governorate) were phage type 40.³

F.- Salmonella gallinarum-pullorum

230 strains of Salmonella gallinarum-pullorum among which 265 provided by the International Salmonella Center, Institut Pasteur, Paris, and 15 locally isolated in Egypt were under lysogenic study. This work has allowed for the isolation of 12 phages.

The routine test dilution for these phages has been determined. 11 patterns were found by the use of these phages in their predetermined routine test dilutions. The results are shown by the following table:

³Hablas, R.M., L.M. Abdel-Naby, and M.S. Sallam. 'Phage Typing of Salmonella typhi-murium Related to Two Outbreaks of Food Poisoning in Egypt.' Al-Azhar Medical Journal. (In Press).

Phage Patterns of Salmonella gallinarum-pullorum

Pattern	P H A G E S											
	1	2	3	4	5	6	7	8	9	10	11	12
1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1
2	-	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1
3	C1	C1	C1	C1	C1	C1	C1	C1	-	C1	C1	C1
4	-	C1	C1	C1	C1	C1	C1	C1	-	C1	C1	C1
5	-	C1	C1	C1	-	-	C1	C1	-	C1	C1	C1
6	-	-	-	-	-	-	C1	C1	C1	C1	C1	C1
7	-	-	-	-	-	-	C1	C1	-	C1	C1	-
8	-	C1	-	-	-	C1	-	-	-	C1	-	-
9	C1	-	-	-	C1	-	-	-	-	-	-	-
10	-	-	-	-	C1	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-

II.- Shigella

200 children suffering from diarrhea had been bacteriologically investigated in a combined work with the Pediatric Department, Ain-Shams University, entitled "Bacteriology of Diarrhea in Egyptian Children".

For the isolation of Escherichia coli the samples of stools were plated directly on MaConkey agar. On the other hand, for the isolation of other pathogenic organisms the samples of stools were also inoculated on Selenite broth, incubated at 37°C for 16-18

hours, followed by subculturing on different selective media, namely; S.S. agar, and Desoxycholate Citrate agar.

The suspicious organisms were morphologically, biochemically, and serologically identified.

The frequencies of the isolated micro-organisms were as follows:

<u>Type of Organism</u>	<u>No. of Isolates</u>	<u>Percentage</u>
Nonagglutinable Escherichia coli below 2 years	68	34.00%
Agglutinable Escherichia coli	57	28.50%
Poly 1+V only	11	5.50%
O:86 K61 (B7)	9	4.50%
O:72 K80 (B-)	7	3.50%
O:26 K60 (B6)	6	3.00%
O:44 K74 (L)	5	2.50%
O:111 K58 (B4)	4	2.00%
O:114 K-(B-)	4	2.00%
O:55 K59 (B5)	3	1.50%
O:25 K11 (L)	2	1.00%
O:126 K71 (B16)	2	1.00%
O:127 K63 (B8)	1	0.50%
O:128 K67 (B12)	1	0.50%
O:119 K69 (B14)	1	0.50%
O:124 K72 (B17)	1	0.50%
Proteus	19	9.5%
Proteus mirabilis	12	6.00%
Proteus vulgaris	4	2.00%
Proteus morgani	3	1.50%
Shigella	18	9.00%
Shigella flexneri	8	4.00%
Shigella dysentery	5	2.50%
Shigella boydii	2	1.00%
Shigella sonnei	3	1.50%
Klebsiella	10	5.00%
Citrobacter	4	2.00%
pyocyanous	2	1.00%
Salmonella typhi Vi-	2	1.00%

In addition to 12 rough Escherichia coli and 8 commensal Escherichia coli which, both, represent 10% of the studied strains.

The two strains of Salmonella typhi isolated in this work have not been subjected to phage typing as they are Vi-.

Phage typing of Shigella sonnei isolated will be carried out and reported in the next season.

III.- Bacillus pyocyaneus

20 phages recently supplied by the Cross-Infection Reference Laboratory, Central Public Health Laboratory, Colindale, London, are being used for the phage typing of the Bacillus pyocyaneus collection available from different sources.

IV.- Neisseria meningitidis

Due to the fact that meningococcal meningitis cases were very rare during this last season, work with Neisseria meningitidis will be resumed next season.

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OFFICE OF NAVAL RESEARCH
Contract Number N00014 - 73 - C - 0009

Annual Report Number IV
(April 1976 - March 1977)

"Lysogeny and Bacteriocinogeny in Salmonella, Shigella, Bacillus
pyocyaneus and Neisseria meningitidis"

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82 04 15 024

"Lysogeny and Bacteriocinogeny in Salmonella, Shigella,

Bacillus pyocyaneus and Neisseria meningitidis"

I.- Salmonella

A.- Salmonella typhi

A total number of 90 strains of S. typhi was isolated this year in the amounts of 67 in Cairo and 23 in Alexandria.

Phage typing of strains isolated in Cairo gave the following results:

Phage typing of S. typhi in Cairo

<u>Phage type</u>	<u>No. of Strains</u>	<u>Percentage</u>
Degraded	14	20.9%
I + IV	14	20.9%
46	10	14.9%
N.T.	8	11.9%
T	6	8.9%
C ₁	5	7.5%
A	3	4.5%
E ₁	3	4.5%
C ₂	1	1.5%
F ₃	1	1.5%
G ₁	1	1.5%
40	1	1.5%
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TOTAL	67	100.0%

Phage typing distribution of strains isolated in Alexandria shows the following:

Phage typing of S. typhi in Alexandria

<u>Phage type</u>	<u>No. of Strains</u>	<u>Percentage</u>
N.T.	8	34.9%
T	3	13.1%
46	3	13.1%
C ₁₀	2	8.8%
A	1	4.3%
C ₁	1	4.3%
D ₁	1	4.3%
D ₄	1	4.3%
E ₁	1	4.3%
I + IV	1	4.3%
Degraded	1	4.3%
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TOTAL	23	100.0%

Together, these results show the following distribution of S. typhi phage types in Egypt as represented by the two cities under this study.

<u>Phage type</u>	<u>No. of Strains</u>	<u>Percentage</u>
N.T.	16	18.9%
Degraded	15	16.7%
I + IV	15	16.7%
46	13	14.5%
T	9	10.0%
C ₁	6	5.6%
A	4	4.4%
E ₁	4	4.4%
C ₁₀	2	2.2%
C ₂	1	1.1%
D ₁	1	1.1%
D ₄	1	1.1%
F ₃	1	1.1%
G ₁	1	1.1%
40	1	1.1%
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TOTAL	90	100.0%

Chemotyping of these strains showed that 73 were of chemotype I and 17 of chemotype II in the percentages of 81.1 and 18.9, respectively.

Also 10 among these strains were colicin positive whereas 80 were colicin negative.

B.- Salmonella paratyphi A

Isolation of 152 strains of S. paratyphi A in the numbers of 127 in Cairo and 25 in Alexandria, and their phage typing have yielded the following results:

Phage typing of S. paratyphi A in Cairo

<u>Phage type</u>	<u>No. of Strains</u>	<u>Percentage</u>
1	111	87.4%
N.T.	7	5.5%
Rough	4	3.1%
2	3	2.4%
4	2	1.6%
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TOTAL	127	100.0%

Phage typing of S. paratyphi A in Alexandria

<u>Phage type</u>	<u>No. of Strains</u>	<u>Percentage</u>
1	20	80.0%
4	4	16.0%
2	1	4.0%
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TOTAL	25	100.0

These two sets of results lead to the following distribution in Egypt:

<u>Phage type</u>	<u>No. of strains</u>	<u>Percentage</u>
1	131	86.2%
N.T.	7	4.6%
4	6	4.0%
2	4	2.6%
Rough	4	2.6%
	<hr/>	<hr/>
TOTAL	152	100.0%

N.B. The results of phage typing of S. typhi and S. paratyphi A in Alexandria, during the period January 1974 to December 1975, show in the article by Lackany et al.[■]

C.- Salmonella paratyphi B

3 strains of S. paratyphi B were isolated from children complaining of diarrhoea associated with measles. These strains will be phage typed and reported in the next period.

D.- Salmonella typhi - murium

Phage types of 4 strains of S. typhi-murium, 3 isolates from chicken and 1 from calf stool, will be identified and reported in the next due report.

■ Lackany, A.S., Wahdan, M.H., Sallam, S.A., Hassan, M.N. and Hablas, R. "Familial spread of enteric fever in Alexandria - Egypt" J. Egypt. Publ. Hlth. Assoc. (in press).

F.- Newly isolated Salmonella in Egypt:

The first occurrence of Salmonella hadar (6, 8: Z₁₀: e, n, x) in Egypt has been recorded when isolated from the stool of an adult patient suffering from enteric fever. This adds to the Salmonella serotypes newly isolated in Egypt mentioned in the work of Hablas et al (1973).[■]

G.- Miscellaneous Salmonella Serotypes

An isolate from human stool proved to be Salmonella muenchen (6, 8: d: 1, 2). While 5 other strains isolated from calf stool were:

- 3 Salmonella carraw (6, 14, 24: y: 1, 7);
- 1 Salmonella muenchen (6, 8: d: 1, 2);
- 1 Salmonella newport (6, 8: e, h: 1, 2);

A part from an isolate of Salmonella group C₁ from a child complaining of diarrhoea associated with measles. Complete serological identification of this culture is being carried out.

■ R. Hablas, W.R. Sanborn and K. Sorensen. "Salmonella Serotypes Newly Isolated from Humans in Egypt". Tropical and Geographical Medicine. 26 (1974) 84 - 86.

H.- Salmonella gallinarum-pullorum*

The 280 strains of *S. gallinarum-pullorum* among which 265 were provided by the International Salmonella Center, Institut Pasteur, Paris, and 15 isolated locally, were phage typed by use of the 12 isolated lysogenic phages in their routine test dilution. The results of the different types are tabulated:

Phage type	Strains		Total	Percentage
	Local strains	International strain		
1	-	13	13	4.6
2	2	35	37	13.2
3	-	6	6	2.1
4	-	7	7	2.5
5	-	4	4	1.4
6	1	4	5	1.8
7	6	2	8	2.9
8	-	1	1	0.4
9	-	12	12	4.3
10	5	142	147	52.5
Non typable	1	39	40	14.3
TOTAL			280	100.0

Photos of the eleven patterns of reactions are enclosed.

* Mohamed M. Ismail, M.D. Thesis "Establishment of scheme of phage typing of *Salmonella gallinarum-pullorum* group by study of lysogeny in different strains isolated from different sources and localities," (In Preparation).

II.- Shigella

A thesis studying incidence of Shigella serotypes in patients suffering from diarrhoea with special reference to their immunologic features is being carried out, its results will appear in the next report.

Shigellosis as a cause of diarrhoea in Egyptian children has been reported by Khalil et al.¹

III.- B. pyocyaneus ²

Ninety two collected cultures of B. pyocyaneus from inpatients and environments of different wards, in Al-Zahraa and Bab-El-Shaaria hospitals, were subjected to pyocine typing following the technics described by Gillies and Govan (1966). The culture medium used was that recommended by Darrell and Wahba (1964).

Pyocine typing of these strains is considered to be the first marker to trace the source of infection. The other important tools i.e. serotyping and phage typing are being carried out in order to have more data for the completion of this epidemiological study.

¹ K.A.H. Mourad, R. Hablas, S.M. Abdel-Pattah, S. El-Sokary, G. Abdel-Khalek, M. Fauzi. Bacteriology of Diarrhoea in Egyptian Children. J.Egypt. Assoc. Ped.

² Effat, M.D. Thesis "Cross hospital infections". (In preparation).

J. H. Darrell and A.H. Wahba Pyocine typing of hospital strains of Ps. pyocyanea. J. of clin. Path. Vol. 17. 236(1964).

R.R. Gillies and J.R. Govan. Typing of Ps. pyocyanea by pyocine Production. J. path. Bact. 91: 339-345 (1966).

Results of Pyocine typing

Pyocine Types	Al-Zahraa Hospital		Babel Shaaria Hospital		Total	Percentage
	No. of Patients	Environment	No. of patients	Environment		
Surgical Wards	1	11	2	1	19	20.65
	10	-	-	-	1	1.10
	3	1	-	-	1	1.10
	27	-	-	-	1	1.10
	30	1	-	-	1	1.10
	35	2	-	-	2	2.20
Non-typable (N.T.)		12	1	-	14	15.20
Urinary Wards	1	4	1	1	8	8.70
	6	6	-	-	9	9.80
	10	-	1	-	3	3.25
	N.T.	1	2	-	3	3.25
E.N.T. Wards	1	1	1	-	2	2.20
	6	2	-	-	4	4.30
	10	2	-	-	3	3.25
	35	-	-	-	1	1.10
	N.T.	2	-	-	3	3.25
Burn Ward	1	9	3	-	12	13.00
	6	2	-	-	2	2.20
	N.T.	2	1	-	3	3.25
TOTAL		58	12	2	92	100.00

BACTERIAL TYPING PROJECT

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OFFICE OF NAVAL RESEARCH
Contract Number N00014 - 73 - C - 0009

Annual Report Number V
(April 1977 - March 1978)

"Lysogeny and Bacteriocinogeny in Salmonella, Shigella, Bacillus
pyocyaneus and Neisseria meningitidis"

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82 04 15 025

"Lysogeny and Bacteriocinogeny in Salmonella, Shigella,
Bacillus pyocyaneus and Neisseria meningitidis"

I.- Salmonella

A.- Salmonella typhi

During this reporting period phage typing of a total number of 70 cultures of Salmonella typhi, isolated in Cairo, has been performed.

The technique of Craigie and Felix still being utilised using the phages obtained from the "Enteric Reference Laboratory, Colindale, London".

Phage typing of S. typhi in Cairo

<u>Phage type</u>	<u>No. of Strains</u>	<u>Percentage</u>
Degraded	22	31.42
I + IV	19	27.15
46	8	11.42
Non-typable	8	11.42
E ₁	6	8.57
C ₂	4	5.75
A	2	2.85
J ₁	1	1.42
Total	<u>70</u>	<u>100.00</u>

Chemotyping of these isolates by the utilisation of Christensen technique revealed that 70% were chemotype I and 30% chemotype II.

Colicin study on the indicator E. coli K₁₂ showed that 85.70% were negative and 14.30% were positive.

B.- Salmonella paratyphi A

Banker phage typing of 141 strains of Salmonella paratyphi A, isolated in Egypt, showed the following results:

<u>Phage type</u>	<u>No. of Strains</u>	<u>Percentage</u>
1	113	80.14
Non-typable	11	7.80
4	10	7.10
2	7	4.96
	<hr/>	<hr/>
Total	141	100.00
	====	=====

N.B. Results of phage typing of S. paratyphi A strains originating from Indonesia, previously reported in our Semi - Annual Report No. IV, have recently appeared in J. Hyg., Camb. (1977), 79, 1.

C.- Phage restriction of *S. typhi* and *S. paratyphi* A

Phage restriction in *S. typhi* and *S. paratyphi* A by ? and resistance factors in *E. coli* has been studied. The aquisition of *S. typhi* Vi phage type "A" (Enteric Reference Lab. E.R.L. No. 26 R:862) to transfer factor of *E. coli* K₁₂ (E.R.L. No. 18 R:312) lead to restriction of multiplication of Vi phages, while the aquisition of the same *S. typhi* of resistance to tetracycline from *E. coli* K₁₂ (R:113) lead to reactions against some bacteriophages which were impossible to interpret, i.e. "Degraded".

On the other hand transfer of resistance factor from *E. coli* (R:tetra. chlor. sulph.) to the same *S. typhi* and *S. paratyphi* A phage type 1 had not changed their phage patterns.

N.B. M.D. thesis (Under preparation).

D.- Antibiotic sensitivity patterns of Egyptian Salmonella serotypes

Antibiogram was performed, according to the method of Bauer et al. (1966), for 131 strains of *S. paratyphi* A, 83 strains of *S. typhi*, 3 strains of *S. montevideo* and one strain of *Salmonella* group C₂. Resistance to

antisalmonella drugs was not observed except in one S. paratyphi A and the 3 S. montevideo which proved to be multiply resistant including chloramphenicol and/or ampicillin. This multiply resistance was found to be chromosomal as it was neither transferred to E. coli K₁₂ F⁻(R:Az 115), nor eliminated by acriflavine.

N.B. M.D. thesis (Under preparation).

E.- Salmonella typhi-murium

Fourteen cultures of S. typhi-murium isolated from different sources, chicken, calf, etc. are being subjected ~~to~~^{to} phage typing.

F.- Salmonella serotypes newly isolated in Egypt

Seven serotypes of Salmonella were isolated for the first time in Egypt. These pathogens were isolated from the stools of healthy or diseased buffalo calves: 3 stains of S. carrau (6, 14, 24:y: 1, 7): 3 strains of S. infantis (6,7 :r: 1,5): 3 strains of S. meleagridis (3,10 :e,h: 1,w): one strain from each S. muenchen (6,8 :e,h: 1,5): S. ferruch (8 :e,h: 1,5) and S. newport

BACTERIAL TYPING PROJECT

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OFFICE OF NAVAL RESEARCH
Contract Number N00014 - 73 - C - 0009

Annual Report Number VI
(April 1978 - March 1979)

"Lysogeny and Bacteriocinogeny in Salmonella, Shigella, Bacillus
pyocyaneus and Neisseria meningitidis"

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82 04 15 026

"Lysogeny and Bacteriocinogeny in Salmonella, Shigella,
Bacillus pyocyaneus and Neisseria meningitidis"

I.- Salmonella

A.- Salmonella typhi

A total number of 38 cultures of S. typhi isolated in Cairo were subjected for phage typing. This has been performed by the use of phages obtained from the "Enteric Reference Laboratory, Colindale, London" following the technique of Craigie and Felix.

Phage typing of S. typhi

<u>Phage type</u>	<u>No. of Strains</u>	<u>Percentage</u>
Degraded	10	26.32
I + IV	9	23.68
Non-typable	8	21.05
46	4	10.53
E ₁	3	7.90
A	2	5.26
J ₁	1	2.63
T	1	2.63
Total	<u>38</u>	<u>100.00</u>

Study of production of colicin by these 38 S. typhi strains on the indicator E. coli K₁₂ revealed that 31 (81.6%) were negative and 7 (18.4%) were positive.

Christensens chemotyping of these cultures showed that 34 strains (89.5%) of chemotype I and 4 strains (10.5%) of chemotype II.

B.- S. paratyphi A.

Excluding 12 rough strains 12 cultures of S. paratyphi A isolated over the last year have revealed the following types using Banker's phage typing method;

<u>Phage type</u>	<u>No. of Strains</u>	<u>Percentage</u>
1	8	66.67
2	3	25.00
4	1	8.33
Total	<u>12</u>	<u>100.00</u>

C.- Salmonella typhi-muruim

Bacteriophages utilised for phage typing of S. typhi-muruim have been recently received from "Enteric Reference Laboratory, Colindale, London."

Thirty nine cultures of S. typhi-muruim isolated from different sources rodents, chicken, calves etc. are being subjected for phage typing following Anderson's system.

N.B. Frequencies of phage types of Egyptian isolates of S. typhi, S. paratyphi A and S. typhi-murum, obtained from our project, compared by previous findings of other authors were presented in the "W.H.O. Regional Scientific Meeting on the Epidemiology of Enteric Infections at Alexandria." in May 1978.

D.- Salmonella gallinarum-pullorum

A field trial for application of phage typing scheme for epidemiological purpose was tried by the use of 12 phages prepared from lysogenic strains of S.gallinarum-pullorum obtained from International Salmonella Center, Paris.

Specimens were collected from 900 dead and affected ducks obtained from a governmental farm at Cairo during 1977. In addition 50 samples of ration for feeding these flocks were also examined.

Thirty S. pullorum strains were isolated from 900 dead and diseased ducks, in addition 4 S. pullorum strains were isolated from 50 ration samples. Results of phage typing of these 34 S. pullorum strains is illustrated in the following table.

<u>Phage type</u>	<u>Dead and Diseased Ducks</u>	<u>Ration</u>
1	11	-
6	19	3
7	-	1
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Total	30	4
	<hr/>	<hr/>

E.- Salmonella serotypes newly isolated in Egypt.

In addition to the seven salmonella serotypes forwarded in the last annual report S. ohio (6,7: b :1,w) has been isolated, for the first time in Egypt from the stools of a child suffering from measles associated with diarrhea. The serological identification was confirmed by the International Salmonella Center, Pasteur Institute, Paris.

II.- Bacillus pyocyaneus.

Chronic otitis media is a frequent disease in Egypt. Bacillus pyocyaneus is the most common organism isolated from the chronic discharging ears. Most isolates are multiply resistant to chemotherapeutic agents.

M.D. "Ear, Nose and Throat" (E.N.T.) thesis has been recently recorded for the evaluation of the role of bacteriophages as therapeutic agents in the treatment of patients suffering from Bacillus pyocyaneus chronic otitis media.

BACTERIAL TYPING PROJECT

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Contract Number N00014 - 73 - C - 0009

**Semi - Annual Report Number VII
(April 1979 - September 1979)**

**"Lysogeny and Bacteriocinogeny in Salmonella, Shigella, Bacillus
Pyocyaneus and Neisseria meningitidis"**

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82 04 15 027

"Lysogeny and Bacteriocinogeny in Salmonella, Shigella,
Bacillus pyocyaneus and Neisseria meningitidis"

Salmonella

A combination research, field work with NAMRU 3, Cairo is being carried out to study the incidence of different Salmonella serotypes in Upper Egypt. The study includes four towns belonging to Quina Governorate namely Quina, Luxor, Asna and Nagaa Hamady.

A- Salmonella typhi

During this reporting period the total of 85 cultures of S. typhi Vi⁺, isolated from the above mentioned localities, were subjected for phage typing following the technique of Craigie and Felix.

Phage types of S. typhi

Phage types	No. of strains	Percentage	D I S T R I B U T I O N			
			Quina	Luxor	Asna	N. Hamady
Non-typable	23	27.1	9	11	3	0
Degraded	14	16.5	6	4	1	3
29	10	11.8	6	0	0	4
A	9	10.6	3	6	0	0
T	9	10.6	3	1	5	0
C ₁	8	9.4	7	1	0	0
46	4	4.7	3	1	0	0
B ₂	2	2.3	1	0	1	0
D ₆	2	2.3	1	0	0	1
G ₁	2	2.3	0	0	2	0
48	1	1.2	0	0	0	1
49	1	1.2	1	0	0	0
Total	85	100.0	40	24	12	9

Kristensen chemotyping of these strains of S.typhi revealed that 72 were of chemotype I and 13 of chemotype II in the percentage of 84.7 and 15.3 respectively.

Also 5(5.9 %) among these strains were colicin producers whereas 80 (94.1 %) were colicin negative.

B- Salmonella Paratyphi A

Isolation of 77 cultures of S.paratyphi A in the numbers of 42 in Quina, 19 in Luxor, 9 in Asna and 7 in Nagaa Hamady and their phage typing ,by the use of Banker's phages, yielded the following results:

Phage types of S.paratyphi A

Phage types	No.of strains	percentage	D I S T R I B U T I O N			
			Quina	Luxor	Asna	N.Hamady
1	44	57.1	28	10	2	4
2	17	22.1	11	5	0	1
Non-typable	14	18.2	3	4	7	0
4	2	2.6	0	0	0	2
Total	77	100.0	42	19	9	7

C- Salmonella typhi-murium ②

A total of 47 cultures of S.typhi-murium were isolated from ducklings, duck eggs, ducks, and rations in Bahtim duck farm. These isolates were phage typed by the utilisation of standard phages obtained from "Enteric Reference Laboratory" Colindale, London following Anderson's scheme.

Phage types of S.typhi-murium

Phage types	No.of strains	Percentage	D I S T R I B U T I O N			
			Ducklings	Duck eggs	Ducks	Rations
Non-typable	40	85.1	27	6	4	3
151	5	10.6	0	2	3	0
8	2	4.3	1	0	0	1
Total	47	100.0	28	8	7	4

② V.D. Thesis ,Cairo University.